

# Inflammatory molecules might become both biomarkers and therapeutic targets for stroke management

Laura Ramiro , Alba Simats, Teresa García-Berrocso and Joan Montaner

**Abstract:** Stroke is the fifth leading cause of death and the most frequent cause of disability worldwide. Currently, stroke diagnosis is based on neuroimaging; therefore, the lack of a rapid tool to diagnose stroke is still a major concern. In addition, therapeutic approaches to combat ischemic stroke are still scarce, since the only approved therapies are directed toward restoring blood flow to the affected brain area. However, due to the reduced time window during which these therapies are effective, few patients benefit from them; therefore, alternative treatments are urgently needed to reduce stroke brain damage in order to improve patients' outcome. The inflammatory response triggered after the ischemic event plays an important role in the progression of stroke; consequently, the study of inflammatory molecules in the acute phase of stroke has attracted increasing interest in recent decades. Here, we provide an overview of the inflammatory processes occurring during ischemic stroke, as well as the potential for these inflammatory molecules to become stroke biomarkers and the possibility that these candidates will become interesting neuroprotective therapeutic targets to be blocked or stimulated in order to modulate inflammation after stroke.

**Keywords:** biomarkers, cerebrovascular disease, inflammation, neuroprotection, stroke, therapeutic targets

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## Introduction

Stroke is caused by the disruption of the blood flow into a brain region and the consequent oxygen and nutrient deprivation, resulting in cell death and severe brain damage. It is the fifth most common cause of death and the most frequent cause of permanent disability in adults worldwide. Ischemic stroke, which is caused by the obstruction of a blood vessel by a thrombus, represents 87% of all strokes.<sup>1</sup>

After years of in-depth research and despite advances in the understanding of stroke pathophysiology, the therapeutic options for acute stroke remain very scarce. Currently, the only approved therapy for treating acute ischemic stroke is the administration of intravenous recombinant tissue-plasminogen activator (rt-PA)

together with endovascular mechanical thrombectomy to remove the thrombus. However, rt-PA treatment carries a significant risk of secondary bleeding.<sup>2</sup> Furthermore, it has a reduced time window of action, given that it is effective only when administered within 4.5 h after the onset of symptoms.<sup>3</sup> This limitation reduces the number of stroke patients who can be treated to approximately 15%.<sup>4</sup> Although mechanical thrombectomy has recently demonstrated its efficacy even 24 h after the onset of the cerebrovascular event,<sup>5</sup> neuroprotective therapies are sought in order to rescue the compromised tissue in the peri-infarct zone of the brain. Although different neuroprotective therapies have shown good results in experimental models, none of them has achieved good results at the clinical level. For that reason, alternative therapies are urgently needed to combat

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Correspondence to:

**Joan Montaner**  
Neurovascular Research  
Laboratory, Vall d'Hebron  
Institute of Research, Pg.  
Vall d'Hebron 119–129,  
Hospital Universitari  
Vall d'Hebron, 08035  
Barcelona, Spain  
[joan.montaner@vhir.org](mailto:joan.montaner@vhir.org)

**Laura Ramiro**  
**Alba Simats**  
**Teresa García-Berrocso**  
Neurovascular Research  
Laboratory, Vall d'Hebron  
Institute of Research,  
Universitat Autònoma de  
Barcelona, Barcelona,  
Spain

stroke. In addition, stroke diagnosis is currently based on medical history, neurological exploration and neuroimaging; therefore, there are no rapid diagnostic tools, such as a blood test, available in clinics. Such a test would help accelerate stroke treatment and optimize patients' management to ameliorate stroke outcomes.

Given the complexity of stroke, different mechanisms are thought to be involved in its pathophysiology. In fact, increasing evidence shows that inflammation plays an important role in the progression of stroke.<sup>6</sup> For that reason, therapeutic targeting of postischemic inflammation in acute stroke has gained interest as a potential neuroprotective strategy. Here, we provide an overview of the inflammatory processes occurring during ischemic stroke, the potential for these inflammatory molecules as stroke biomarkers and the possibility that these biomarkers will become important therapeutic targets to be blocked or stimulated in order to modulate inflammation after stroke. To that end, studies in which pharmacological agents are specifically directed to modulate inflammatory molecules in the context of stroke will be reviewed.

In our attempt to focus on translational evidence as much as possible, studies including knockout animals for inflammation-related genes are considered beyond the scope of this review. In addition, studies indirectly modulating inflammatory molecules will not be considered either, given that we want to describe the specific role of each individual protein in the pathophysiology of ischemic stroke.

### Postischemic stroke inflammation

After the ischemic event, the blockage of blood flow produces a reduction of glucose and oxygen availability, which leads to an impaired adenosine triphosphate production and ultimately causes a bioenergetics failure.<sup>7</sup> In addition, an ionic imbalance occurs within minutes after the arterial occlusion, generating a reduced reuptake of glutamate and subsequently leading to excitotoxicity and apoptosis. At the same time, catabolic enzymes are activated, producing an increase in the generation of reactive oxygen species. This fact triggers the expression of pro-inflammatory genes, such as cytokines and chemokines, by the injured brain cells.<sup>8</sup> Consequently, the expression of cell adhesion molecules on the endothelial cell surface is

induced, including P-selectin, E-selectin and intercellular adhesion molecule-1 (ICAM-1). These molecules mediate the infiltration of leukocytes into the brain parenchyma and facilitate the clearance of debris in the infarct area. Moreover, endothelial cells increase the expression of chemokines in order to guide leukocytes to the site of injury.<sup>9</sup> However, there is evidence showing that beside their beneficial role, infiltrating immune cells also impair the ischemic brain by producing cytotoxic mediators that can extend the inflammatory response and increase brain damage.<sup>10</sup>

In addition, dying brain cells secrete damage-associated molecular patterns (DAMPs) that are recognized by microglia and cause their activation. Thus, both resident and infiltrated immune cells produce a burst of pro-inflammatory molecules within the infarct area.<sup>11</sup> This fact couples with an increase in matrix metalloproteinase (MMP) production, which mediates the destruction of the basal lamina, increasing blood-brain barrier (BBB) permeability and facilitating the entrance of additional peripheral immune cells into the affected brain area.<sup>12</sup>

Furthermore, the complement cascade, a major constituent of innate immunity, has been shown to play an important role in stroke. The activation of the complement system is mediated by an increased expression of complement cascade receptors and activators (C1q and C3) by damaged cells in the site of injury. Its activation generates opsins (C3b, C4b and C5b), which enhance the phagocytic response, as well as inflammatory mediators and a membrane attack complex (C5b, C6, C7, C8 and C9) that has cytolytic activity.<sup>13</sup> In addition, complement system activation has been implicated in adhesion molecule upregulation, chemotaxis and leukocyte activation.<sup>14</sup>

Acute stroke not only causes a local inflammatory response in the ischemic brain but also triggers a systemic immune response. After the ischemic event, there is a major release of pro-inflammatory mediators into the systemic circulation, causing an overactivation of peripheral immune cells. This excessive activation leads to an exhaustion of mature leukocytes, provoking the recruitment of immature leukocytes that are unable to respond properly to brain damage.<sup>15</sup> In fact, the recruitment and expansion of this immature subpopulation ultimately lead to lymphocytopenia, contributing to a significant immunodepression that predisposes

patients to poststroke infections and influence stroke outcome.<sup>16</sup> Furthermore, the disproportionate concentration of pro-inflammatory mediators can stimulate the activation of the hypothalamic–pituitary–adrenal axis and the sympathetic nervous system, with the consequent release of catecholamines and glucocorticoids. All this leads to the inhibition of pro-inflammatory pathways and the stimulation of anti-inflammatory mechanisms through the release of interleukins and growth factors. In addition, this immunosuppression helps prevent autoimmune reactions against the central nervous system (CNS) antigens that are present in the bloodstream due to BBB leakage.<sup>17</sup>

Finally, in the brain, the immune system also works to terminate the inflammatory response at later stages. To that end, infiltrating macrophages and activated microglia are known to phagocytose dead cells and debris. Moreover, they promote the production of anti-inflammatory molecules that contribute to the suppression of the immune response, and at the same time, inhibit the expression of adhesion molecules and the production of pro-inflammatory cytokines.<sup>6</sup> In addition, both macrophages and microglial cells release neuroprotective factors required for the recovery of the ischemic brain injury that promote neurogenesis and angiogenesis, among other processes.<sup>18</sup> Figure 1 summarizes the postischemic inflammatory cascade, highlighting key proteins that play a relevant role in the process.

### Biomarkers in stroke

Biomarkers are defined as characteristics that can be objectively measured and evaluated as indicators of normal biological processes, pathogenic processes, or pharmacologic responses to therapeutic interventions.<sup>19</sup> Clinical biomarkers can be detectable molecules from different biological fluids such as blood, urine, or saliva. Ideally, biomarkers should have high specificity and sensitivity for the indicated process, and their measurements should be accurate, reproducible, and easily interpretable by clinicians. In terms of clinical application, stroke biomarkers are considered relevant tools for different phases, including both the diagnosis and the prognosis of stroke. These biomarkers should be representative of the brain injury processes triggered by stroke. As previously described, BBB disruption favors the release of brain antigens into the peripheral circulation, making these molecules promising

stroke-associated biomarkers that could reflect in the circulatory system those pathological processes occurring in brain after the ischemic event. In addition, both local and peripheral inflammatory response can produce systemic indicators that might serve as stroke biomarkers.

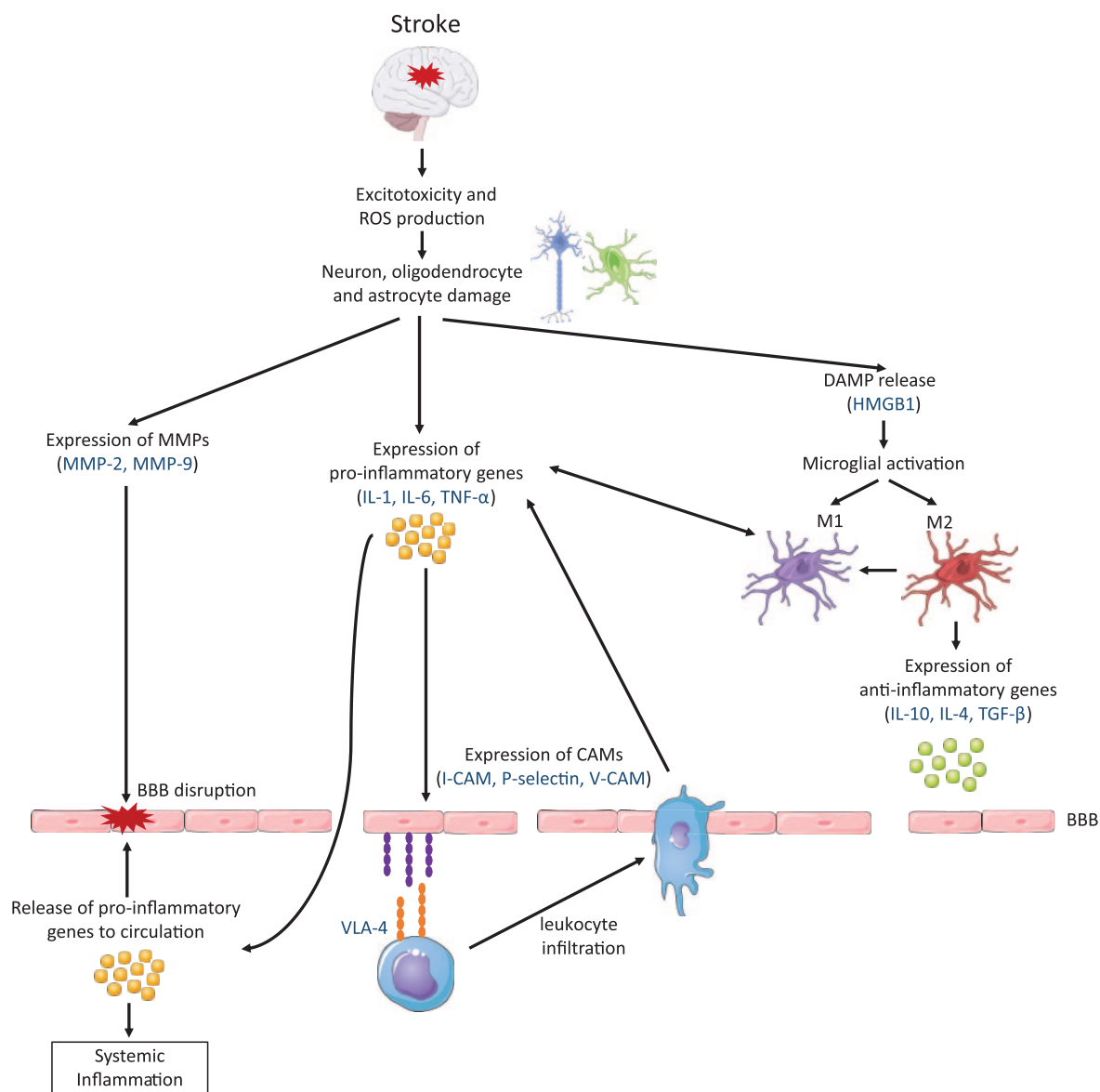
Different inflammation-related proteins have been found altered after ischemic stroke and have shown a potential role in the detection and modulation of inflammation, making them potential biomarkers or therapeutic targets for stroke management. Those being studied for both roles will be reviewed in the following sections. Table 1 summarizes the main studies performed for each of the proteins described in this review.

### Main inflammatory players in stroke

#### *Cytokines*

Cytokines are small proteins that through extracellular signaling regulate different biological functions such as innate and acquired immunity, inflammation, proliferation and repair. Cytokines have both pro- and anti-inflammatory properties and play an important role in the progression of the stroke-associated inflammation.<sup>152</sup> After the ischemic event, cytokines are upregulated in the brain, expressed by both cells of the immune system and resident brain cells such as neurons and microglia.<sup>153</sup>

*Pro-inflammatory cytokines.* Interleukin-1 (IL-1) is produced in the CNS by microglia, astrocytes, endothelium and neurons. It has two different forms, one intracellular (IL-1 $\alpha$ ) and one secreted (IL-1 $\beta$ ). Both forms act through the IL-1 type I receptor (IL-1RI) which is expressed by immune and endothelial cells. This receptor is competitively blocked by the naturally occurring IL-1 receptor antagonist (IL-1Ra), also secreted by endothelial and immune cells and binds to the receptor without inducing any effect.<sup>154</sup> In reference to the plausible role of the IL-1 family as stroke biomarkers, there is some controversy. Distinct studies revealed that ischemic stroke patients have higher circulating levels of IL-1 $\beta$  within 24 h after the ischemic event when compared with controls, as well as when compared with patients with other neurological conditions such as Alzheimer disease and Parkinson disease.<sup>20–24</sup> However, two other studies have reported that IL-1 $\beta$  levels in serum or plasma are not higher in stroke patients when compared



**Figure 1.** The inflammatory cascade following a stroke. BBB, blood–brain barrier; CAM, cellular adhesion molecule; DAMP, damage-associated molecular pattern; HMGB1, high-mobility group box 1; I-CAM, intercellular adhesion molecule; IL, interleukin; MMP, matrix metalloproteinase; ROS, reactive oxygen species; TGF, transforming growth factor; TNF, tumor necrosis factor; V-CAM, vascular cell adhesion molecule; VLA, very late antigen.

with healthy controls, at 12 h<sup>25</sup> and 72 h after the onset of symptoms.<sup>26</sup> These contradictory results observed among studies might be explained, in part, by the differences in the time of evaluation of the biomarker or the sample size of the stroke patient cohorts (up to 120 for those studies reporting higher levels in stroke patients *versus* less than 50 patients in those reporting no differences). In addition, it has been shown that plasma levels of IL-1Ra are higher in patients than in controls

within the first 24 h and even at 72 h after the ischemic event.<sup>25–27</sup> However, these elevated levels of IL-1Ra may not be sufficiently high to prevent IL-1 activation of receptors in target cells.<sup>27</sup> Several studies have investigated the therapeutic effect of the modulation of this pathway. Various studies have shown that IL-1 $\beta$  administration increases the infarct size in mice after transient middle cerebral artery occlusion (tMCAO)<sup>28</sup> and rats after permanent (pMCAO) or transient ischemia.<sup>33,34</sup>

**Table 1.** Main studies performed on inflammatory-related proteins having an important role in stroke pathophysiology.

Protein	Role as biomarker			Experimental studies			Human clinical trials		
	Type	Blood levels	Intervention	Rodent model	Infarct volume changes	Design	Results		
IL-1 $\beta$ /IL-1Ra	Diagnosis	Higher levels of IL-1 $\beta$ in IS than in controls within 24 h after the event. <sup>20-24</sup> No differences in IL-1 $\beta$ levels between IS and controls at 12 or 72 h after the event. <sup>25,26</sup> Higher levels of IL-1Ra in IS than in controls <sup>25-27</sup>	IL-1Ra/IL-1 $\beta$ administration	tMCAO mouse	IL-1Ra ↓ <sup>28,29</sup> IL-1 $\beta$ ↑ <sup>28</sup>	34 IS: 17 received intravenous IL-1Ra over 3 days and 17 placebo. <sup>30,31</sup>	Drug is safe and well tolerated; it produced a reduction of the inflammatory response (white cell counts and pro-inflammatory proteins) and reversed peripheral immunosuppression; improved 3-month outcome		
				tMCAO rat	IL-1Ra ↓ <sup>32</sup> IL-1 $\beta$ ↑ <sup>33</sup>	80 IS: 39 receiving subcutaneous IL-1Ra for 3 days and 41 receiving placebo <sup>39</sup>	Drug reduces plasma inflammatory markers; no improvement in 3-month outcome		
VCAM-1/ VLA-4	Diagnosis/ prognosis	Higher levels of VCAM-1 in IS than controls; <sup>23,24,40-43</sup> association between higher VCAM-1 levels and worse outcome <sup>44,45</sup>	Anti-VLA-4 administration	pMCAO mouse	No change <sup>46,47</sup> ↓ <sup>46,48,49</sup>	161 IS: 79 received intravenous natalizumab and 82 placebo <sup>50</sup>	Natalizumab did not reduce infarct volume; natalizumab-treated group had better functional outcome than placebo group		
				pMCAO mouse	No change <sup>48</sup> ↓ <sup>47</sup>	270 participants receiving Natalizumab in a low or a high dose, or placebo	Ongoing [ClinicalTrials.gov identifier: NCT02730455]		

*(Continued)*

Table 1. (Continued)

Protein	Role as biomarker			Experimental studies			Human clinical trials	
	Type	Blood levels	Intervention	Rodent model	Infarct volume changes	Design	Results	
ICAM-1	Diagnosis/prognosis	Higher levels of ICAM-1 in IS than controls within 24 h after the event, <sup>21,23,24,53,54</sup> although another study found no difference; <sup>40</sup> one small study showed lower levels of ICAM-1 in IS than in controls; <sup>55</sup> association between higher ICAM-1 levels and larger infarct size and worse long-term outcome, <sup>21</sup> although no association also found <sup>40,56</sup>	Anti-ICAM-1 administration	tMCAO rat	↓ <sup>51,52</sup>	32 IS: several dose ranges of enlimomab within first 24 hours + four daily doses <sup>60</sup>	160 mg on day 1 + 40 mg/day for 4 days produce the desired blood levels (≥10 µg/ml) without increasing adverse effects	
			tMCAO rat	↓ <sup>57-59</sup>	625 IS: 317 received enlimomab and 308 placebo <sup>61</sup>	Enlimomab-treated group had worsening of neurological functions, increased mortality and adverse drug reactions compared with placebo group		
TNF-α	Diagnosis/prognosis	Higher levels of TNF-α in IS than in controls; <sup>21,23,24,62-65</sup> smaller studies showed no differences in TNF-α between IS and controls; <sup>25,26,66,67</sup> associations between higher TNF-α levels and poor outcome <sup>20,62</sup> and larger lesion size <sup>65,68</sup> are not clear enough <sup>26,69,70</sup>	anti-TNF/TNF-bp administration	tMCAO mouse	Anti-TNF ↓ <sup>71</sup>			

Table 1. (Continued)

Protein	Role as biomarker		Experimental studies			Human clinical trials	
	Type	Blood levels	Intervention	Rodent model	Infarct volume changes	Design	Results
				pMCAO mouse	TNF-bp ↓ <sup>72,73</sup>		
				tMCAO rat	Anti-TNF ↓ <sup>74,75</sup>		
				pMCAO rat	Anti-TNF ↓ <sup>76</sup> TNF-bp ↓ <sup>77</sup>		
TGF-β	Diagnosis/ prognosis	Controversial results: lower, <sup>78,79</sup> equal <sup>80,81</sup> or higher <sup>82</sup> levels of TGF-β in IS than controls; association between high late TGF-β levels and large infarct volume and stroke severity <sup>81</sup>	TGF-β/TGF-β antagonist administration	tMCAO mouse	TGF-β ↓ <sup>83</sup>		
				pMCAO mouse	TGF-β ↓ <sup>84</sup>		
				tMCAO rat	TGF-β antagonist ↑ <sup>85</sup>		
MMP-9/ MMP-2	Diagnosis/ prognosis	Higher levels of MMP-9 in IS than controls <sup>86-93</sup> and stroke mimics; <sup>94-96</sup> no differences between stroke patients (ischemic and hemorrhagic) and controls; <sup>97</sup> association between higher MMP-9 levels and worse outcome and larger infarct volumes; <sup>21,68,86,89,93,98-106</sup> association between higher MMP-9 levels and hemorrhagic transformation following rt-PA administration; <sup>92,107-110</sup> lower levels of MMP-2 in IS than controls; <sup>93,102</sup> smaller study showed no differences <sup>92</sup>	Anti-MMP-9 + anti-MMP-2 administration (SB-3CT)	tMCAO mouse	↓ <sup>111</sup>		

(Continued)

Table 1. (Continued)

Protein	Role as biomarker		Experimental studies			Human clinical trials	
	Type	Blood levels	Intervention	Rodent model	Infarct volume changes	Design	Results
IL-6	Diagnosis/prognosis	Higher levels of IL-6 in IS patients compared with controls <sup>20,21, 23,24,26,62,66,67,113-120</sup> and stroke mimics; <sup>94</sup> association between higher IL-6 levels and poor functional outcome, infections <sup>44,121</sup> and larger infarct volumes <sup>122,123</sup>	IL-6 administration	pMCAO mouse	No change <sup>124</sup>		
IL-10	Diagnosis/prognosis	Lower <sup>78,115,117,126,127</sup> or equal <sup>24,128</sup> IL-10 levels when comparing IS and controls; no association between IL-10 and infarct volume <sup>70,129</sup> or stroke outcome; <sup>70, 126,130,131</sup> association between higher levels of IL-10 and infections <sup>132</sup>	IL-10 administration	pMCAO rat	↓ <sup>125</sup>		
IL-4	Diagnosis/prognosis	Higher levels of IL-4 in IS than controls; <sup>116</sup> no association between IL-4 and neurological worsening <sup>69</sup>	IL-4 administration	tMCAO mouse	↓ <sup>136</sup> No change <sup>137</sup>		
P-selectin	Diagnosis	Higher levels of P-selectin in IS than in controls <sup>23,24,138-140</sup>	Anti-P-selectin administration	tMCAO mouse	↓ <sup>141</sup>		
				tMCAO rat	No change <sup>142</sup>		



Table 1. (Continued)

Protein	Role as biomarker		Experimental studies			Human clinical trials	
	Type	Blood levels	Intervention	Rodent model	Infarct volume changes	Design	Results
HMGB1	Diagnosis/ prognosis	Higher levels of HMGB1 in IS than in controls; <sup>86,145-147</sup> association between higher HMGB1 levels and poor outcome; <sup>86,148</sup> although another study found no association <sup>147</sup>	HMGB/anti-HMGB1 administration	pMCAO rat tMCAO rat	↓ <sup>143,144</sup> HMGB1 ↑; <sup>149</sup> anti-HMGB1 ↓ <sup>149</sup>		
ANXA1	Diagnosis	No differences in ANXA1 levels between IS and controls or stroke mimics. <sup>150</sup>	ANXA1/anti-ANXA1	pMCAO rat	ANXA1 ↓; <sup>151</sup> anti-ANXA1 ↑ <sup>151</sup>		

Inflammatory-related proteins are sorted from the most to the least studied.  
 ANXA1, annexin A1; HMGB1, high-mobility group box 1; ICAM-1, intercellular adhesion molecule-1; IL, interleukin; IL-1Ra, interleukin-1 receptor antagonist; IL-1β, interleukin-1 beta; IS, ischemic stroke patients; MMP, matrix metalloproteinase; pMCAO, permanent middle artery occlusion; rt-PA, recombinant tissue-plasminogen activator; SB-3CT, methylthiirane; TGF-β, transforming growth factor beta; tMCAO, transient middle cerebral artery occlusion; TNF-α, tumor necrosis factor alpha; TNF-bp, tumor-necrosis-factor-binding protein; VCAM-1, vascular cell adhesion molecule-1; VLA-4, very late antigen-4.

On the other hand, the administration of recombinant human IL-1Ra greatly reduces (up to 60%) the infarct volume after MCAO in both mice<sup>28,29</sup> and rats.<sup>32,34–38</sup> In line with this, there are studies showing an improved functional outcome after the administration of IL-1Ra.<sup>155,156</sup> The results obtained in these studies indicate a deleterious role of IL-1 and a plausible mechanism of neuroprotective action through the blockage of this pathway. This has led to the development of clinical trials testing the therapeutic potential of recombinant human IL-1Ra (anakinra) in stroke. In a phase II randomized clinical trial, 34 ischemic stroke patients within 6 h of the onset of symptoms received anakinra (100 mg loading dose over 60 s, followed by a 2 mg/kg/h infusion over 72 h) or placebo, intravenously. This study showed that the drug was safe and well tolerated and that patients receiving anakinra showed a reduced inflammatory response, with lower levels of neutrophils and total white cell counts as well as C-reactive protein (CRP) and IL-6 blood levels. Moreover, although no differences were observed in infarct volumes, 3-month clinical outcome was better in the treated group than in the placebo group.<sup>30</sup> This could be related to the reversal of the peripheral immunosuppression, given that the production of cytokines such as tumor necrosis factor alpha (TNF- $\alpha$ ), IL-6 and IL-10 was reduced in the anakinra-treated group.<sup>31</sup> Very recently, a new phase II trial has explored the subcutaneous administration of IL-1Ra, which increased the half-life of the drug and facilitated its administration. In this study, 39 ischemic stroke patients received a total of six doses of anakinra (100 mg administered twice a day for 3 days) and 49 patients received placebo. The results confirmed that IL-1Ra administration significantly reduced plasma inflammatory markers associated with worse outcome, such as IL-6. However, patients receiving IL-1Ra subcutaneously did not show an improvement on the 3-month outcome when compared with the placebo group.<sup>39</sup> Therefore, although the overall results seem promising, further studies need to be done before its translation to practice. Moreover, it would be interesting to evaluate the efficacy of anakinra on reducing the infarct volume to better understand the role of IL-1 in the stroke pathophysiology.

Interleukin-6 (IL-6) is a pro-inflammatory cytokine that acts as a messenger molecule. It is secreted by different cells including microglia, astrocytes, leukocytes and endothelial cells in response to cerebral injury.<sup>157</sup> Several studies

show that there is an increase in IL-6 circulating levels in stroke patients early after the ischemic event in comparison with controls<sup>20,21,23,24,26,57,62,66,113–120</sup> and even in front of stroke-mimicking conditions such as syncope, seizures, and primary headache disorders.<sup>94</sup> Moreover, high IL-6 levels in blood 24 h after the ischemic event are associated with poor long-term functional outcome<sup>44,121</sup> and with large infarct volumes.<sup>122,123</sup> In addition, it has been shown that an acute increase of IL-6 levels in ischemic stroke patients is associated with poststroke infections within the first week after the event.<sup>121</sup> However, while higher circulating levels of IL-6 are related to morbidity, there is also evidence that IL-6 can have neuroprotective functions after ischemic stroke.<sup>158</sup> For example, it has been described that IL-6 produced by injured brain cells promotes poststroke angiogenesis.<sup>159</sup> For that reason, some studies have explored the neuroprotective role of IL-6 in experimental stroke. One study performed using a pMCAO mouse model showed that IL-6 administration right after reperfusion did not reduce infarct volume but improved functional outcome.<sup>124</sup> Similarly, another independent study performed in a transient tMCAO mouse model showed an improved functional outcome after IL-6 administration.<sup>160</sup> Loddick and colleagues showed that the intracerebroventricular injection of recombinant human IL-6 protein reduced infarct size in rats.<sup>125</sup> In addition, IL-6 prevented learning disability and hippocampal neuron loss when administered in gerbils.<sup>161</sup> All these findings could be indicating different effects of IL-6 between central and systemic inflammation, as well as at different times poststroke. Thus, IL-6 seems to be a good biomarker for stroke diagnosis and prognosis but further studies need to be done to better evaluate its therapeutic effect. Moreover, a blockade of this pathway might also be of interest in order to further evaluate the dual role of this protein.

TNF- $\alpha$  is another important mediator implicated in the pathophysiology of stroke. TNF- $\alpha$  has two forms: a transmembrane form that regulates local inflammation by cell-to-cell interactions, and a soluble form generated by TNF- $\alpha$ -converting enzyme (TACE).<sup>162</sup> The soluble form acts at the systemic level amplifying the phagocytic and cytotoxic action of macrophages and enhancing the expression of other cytokines, such as IL-6 and IL-1. In reference to TNF- $\alpha$  blood levels after ischemic stroke, there are contrasting reports. Some studies have reported that circulating

TNF- $\alpha$  levels do not change after the ischemic event within the first 24 h,<sup>25,26,66,67</sup> whereas others reported an increase in the TNF- $\alpha$  blood levels after stroke in comparison with controls.<sup>21,23,24,62–64,163</sup> However, the lack of differences reported in some studies could be, partly due to a reduced sample size. Specifically, studies reporting no differences include fewer patients (from 19 to 34 ischemic stroke patients) than the ones showing increased TNF- $\alpha$  levels (from 23 to 131 ischemic stroke patients, including 4 studies with more than 100 patients). Moreover, the role of TNF- $\alpha$  as a biomarker for stroke prognosis has also been studied, and contradictory results have also been obtained. Some studies have found an association between higher TNF- $\alpha$  levels and poor outcome<sup>20,62</sup> or larger lesion size.<sup>65,68</sup> However, some other studies have not found these associations.<sup>26,69,70</sup> Thus, further studies need to be done before any conclusions can be drawn about the use of TNF- $\alpha$  as a stroke biomarker. In reference to the experimental models, TNF- $\alpha$  is probably one of the most extensively studied cytokines in the field of stroke research. In a tMCAO mouse model, Yang and colleagues showed that intracerebroventricular injection of an antibody against TNF- $\alpha$  after the brain artery occlusion significantly decreased the infarct volume.<sup>71</sup> Moreover, three independent studies showed that intraperitoneal, intravenous and intracerebroventricular administration of anti-TNF- $\alpha$  in rats decreased the infarct volume using tMCAO<sup>74,75</sup> and pMCAO models.<sup>76</sup> In line with this, other studies showed that either intracranial, intraperitoneal, intravenous or even topic administration of TNF-binding protein (TNF-bp, which binds to and inhibits TNF- $\alpha$ ) decreased the infarct volume following pMCAO in mice<sup>72,73</sup> and when administered intravenously in rats.<sup>77</sup> Finally, Wang and colleagues proved that the administration of a TACE inhibitor reduced the infarct volume in rats.<sup>164</sup> Thus, all these studies seem to indicate that TNF- $\alpha$  has a detrimental role in stroke, since its inhibition seems to be a promising tool for stroke treatment. To corroborate the findings reported in experimental models, it might be interesting to perform a clinical trial in ischemic stroke patients to evaluate the therapeutic potential of inhibiting TNF- $\alpha$ .

*Anti-inflammatory cytokines.* Interleukin-4 (IL-4) is a cytokine produced mainly by leukocytes. Its signaling contributes to a potent anti-inflammatory response through the inhibition of pro-inflammatory cytokines and chemokines, among

other functions.<sup>165</sup> Although it is poorly studied as a stroke biomarker, Kim and colleagues found that acute ischemic stroke patients had higher levels of IL-4 in serum than controls,<sup>116</sup> while similar IL-4 levels were found in ischemic stroke patients with or without neurological worsening.<sup>130</sup> In reference to animal models, functional and cognitive improvement was shown following continuous (starting 6 h after ischemia and lasting for one week) IL-4 administration in a tMCAO mouse model, but without differences in infarct volume when compared with the control group.<sup>137</sup> In contrast, others have found that IL-4 administration decreased infarct volume and improved the behavioral performance and neurological recovery 14 days after stroke in a tMCAO mouse model.<sup>136</sup> Finally, IL-4 administration in rat tMCAO did not reduce the number of degenerating neurons and produced an increase in the number of macrophages/microglia in the infarct zone.<sup>166</sup>

Interleukin-10 (IL-10) is an anti-inflammatory cytokine expressed mainly by monocytes in response to brain injury. It has different immunomodulatory functions during the inflammatory response, being particularly important during the resolution phase. In fact, IL-10 reduces the activation of T cells, macrophages and monocytes, as well as attenuating the synthesis of pro-inflammatory cytokines. Moreover, it also reduces leukocyte adhesion and extravasation through the endothelium.<sup>167</sup> Various studies have measured the circulating levels of this protein after ischemic stroke at different time points. Two studies have reported that patients have lower levels of IL-10 than controls within 12 h after the onset of symptoms.<sup>115,117</sup> However, an independent study reported no differences between patients and controls at this same time point.<sup>24</sup> Moreover, it has also been reported that circulating levels of this protein remain decreased at 48 h,<sup>126</sup> 72 h,<sup>127</sup> and even more than 28 days after the ischemic event.<sup>78</sup> However, a lack of differences in IL-10 circulating levels between patients and controls 72 h after the ischemic event has also been reported.<sup>128</sup> Therefore, the majority of studies seem to indicate a plausible role of IL-10 as a biomarker for stroke diagnosis, but more studies need to be performed. In addition to this, two independent studies revealed that circulating levels of IL-10 were not associated with the initial infarct volume.<sup>70,129</sup> Moreover, some studies have explored the potential of IL-10 as an outcome predictor, although with some controversy as

well. One study found that lower IL-10 levels 24 h after the ischemic event were associated with neurological worsening at 48 h.<sup>130</sup> However, another found that high circulating levels of IL-10 at 48 h after the event were independently associated with severe neurological deficits and predicted 3-month adverse clinical outcome.<sup>126</sup> Another study revealed that those patients who survived after the ischemic event, had decreased levels of IL-10 at 24 h but increased levels of IL-10 at 72 h and at 6 days compared with those patients who died.<sup>131</sup> However, Sahan and colleagues showed no significant association between prognosis and IL-10 levels.<sup>70</sup> In addition to this, some studies showed that patients who developed infections within the first week after the ischemic event had higher IL-10 levels within the first 24 h.<sup>132</sup> Regarding these results, further studies need to be carried before any practical conclusion can be drawn. In reference to experimental studies, two independent studies demonstrated that intracerebroventricular and intravenous administration of IL-10 reduces the infarct volume after pMCAO in rats.<sup>133,134</sup> Moreover, Ooboshi and colleagues reported that postischemic gene transfer of IL-10 into the lateral ventricle reduced the infarct size in a pMCAO rat model.<sup>135</sup> Hence, all experimental studies seem to indicate that the administration of IL-10 has a neuroprotective effect, but its application as a new stroke therapy needs to be further studied before its translation to the clinical setting.

Transforming growth factor beta (TGF- $\beta$ ) is produced by microglia, macrophages and astrocytes after ischemia and it regulates a variety of functions, such as cell growth and differentiation, immune function, and apoptosis.<sup>168</sup> In reference to the differences observed in the circulating levels of this cytokine between ischemic stroke patients and controls, there are contradictory results. Two independent studies reported decreased levels of TGF- $\beta$  in patients after acute ischemic stroke in comparison with controls,<sup>78,79</sup> while two other studies found no differences.<sup>80,81</sup> Moreover, another study reported that TGF- $\beta$  levels were significantly elevated 24 h after the ischemic event.<sup>82</sup> In addition, Stanzani and colleagues reported that higher TGF- $\beta$  levels on the fourth day were associated with larger infarct volumes and an increased severity of ischemic stroke.<sup>81</sup> However, all studies carried out so far have had limited sample sizes, therefore, larger studies need to be undertaken. In addition,

various studies have explored the modulation of TGF- $\beta$  as a potential treatment in experimental stroke. Ma and colleagues reported that the intranasal administration of TGF- $\beta$  produced a significant reduction of the infarct volume in a tMCAO mouse model.<sup>83</sup> Moreover, another study showed that the intracerebroventricular administration of TGF- $\beta$  decreased the lesion size after pMCAO in mice.<sup>84</sup> A different approach was attempted by Ruocco and colleagues, who showed that the administration of a TGF- $\beta$  antagonist increased the infarct volume in a rat tMCAO model,<sup>85</sup> thus demonstrating the beneficial role of TGF- $\beta$  in the ischemic brain.

#### *Cell adhesion molecules*

Cell adhesion molecules (CAMs) play a key role in the trafficking and recruitment of leukocytes to activated endothelia in acute ischemic stroke. In fact, after the ischemic event, there is an increase in CAM expression on the cerebral endothelium. During the progression of inflammation, soluble isoforms of CAMs are shed from the cell surface and released into the bloodstream. CAMs are divided into three groups: the immunoglobulin gene superfamily (ICAM-1 and 2, VCAM-1, PECAM-1 and MAdCAM-1), the selectins (P-, E- and L-selectin), and the integrins (CD11, CD18, CD29 and CD49).<sup>9</sup> Of these, only those CAMs described in the following sections have been studied as both biomarkers and therapeutic targets for stroke.

ICAM-1 is one of the most studied CAMs in the context of ischemic stroke. In reference to changes in the ICAM-1 plasma levels that occur after ischemic stroke, there are contradictory results. Various independent studies reported higher levels of this protein in ischemic stroke patients compared with controls within 24 h after the ischemic event.<sup>21,23,24,53,54</sup> However, another study reported no difference in ICAM-1 levels when comparing patients and controls at the same time point.<sup>40</sup> In addition, reduced levels of this protein at 72 h after the onset of ischemic stroke symptoms were also reported; however, this study included only 14 stroke patients, therefore, these results might not be as robust as those of the other studies, which all included more than 100 ischemic stroke patients.<sup>55</sup> Controversies also exist in reference to ICAM-1 levels and stroke outcome. While two studies reported no association between ICAM-1 levels and stroke severity within the first week after

the event and neither with functional outcome at 10 days or 3 months,<sup>40,56</sup> Sotgiu and colleagues found a significant association with higher admission ICAM-1 levels and worse 3-month outcome, as well as with larger infarct size.<sup>21</sup> In addition, various studies have evaluated the therapeutic potential of anti-ICAM-1 antibody in experimental stroke. Three independent studies reported a reduction of the infarct size after intravenous or intraperitoneal administration of anti-ICAM-1 in a tMCAO rat model.<sup>57–59</sup> However, this treatment seems not to be able to reduce the infarct size after a pMCAO in rats.<sup>58</sup> In addition to this, the inhibition of ICAM-1 as a potential stroke therapy has also been studied at the clinical level. Enlimomab is a murine monoclonal antibody that binds to ICAM-1, inhibiting neutrophil adhesion and migration through the brain endothelium. It was tested for safety, pharmacokinetics and biological activity in 32 stroke patients. The aim of this trial was to identify the dosing regimen that would achieve therapeutic serum levels (above 10 µg/ml), which were based on an *in vitro* study. To that end, a loading dose of enlimomab (ranging between 60 and 160 mg of antibody) was administered within 24 h after the onset of symptoms followed by four daily maintenance doses (ranged between 20 and 80 mg/day). The results obtained revealed that a regimen of initial 160 mg followed by a maintenance dose of 40 mg/day produced the desired blood levels of enlimomab and was well tolerated given that it did not increase the risk of adverse events in stroke patients.<sup>60</sup> Once the safe dosage of the treatment was established, the Enlimomab Acute Stroke Trial (EAST) tested the efficacy of this compound in a total of 625 patients with ischemic stroke (317 received enlimomab and 308 placebo). Unfortunately, the enlimomab-treated group of patients had worsening of neurological functions, an increased mortality and adverse drug reactions (infections and fever) compared with the placebo group, showing the inefficacy of this therapeutic drug in stroke patients.<sup>61</sup> The negative results obtained in this trial have been attributed to a detrimental immunoinactivation due to the administration of a mouse antibody. In fact, Furuya and colleagues reproduced this design in an experimental study in which murine antibodies to ICAM-1 were serially administered to rats. They found that serial administration of the antibody-sensitized rats to produce antimouse antibodies, covered up any potential benefit from the inhibition of leukocyte infiltration to the site of injury.<sup>169</sup>

Another important CAM in the stroke pathophysiology is vascular cell adhesion molecule-1 (VCAM-1), also known as CD106. VCAM-1 is a protein that is expressed on the surface of endothelial cells and mediates cell-to-cell recognition and adhesion, as well as the activation and subsequent passage of leukocytes into the inflamed region.<sup>65</sup> The major ligand of VCAM-1 is very late antigen-4 (VLA-4), which is an integrin constitutively expressed on the membrane of leukocytes. In reference to the plasma levels of these proteins, the circulating levels of VLA-4 in ischemic stroke patients have not been studied for the moment. In addition, several distinct studies have found that soluble VCAM-1 levels in circulation are higher after stroke than in stroke-free controls.<sup>23,24,41–43</sup> Nonetheless, it seems that these increased VCAM-1 levels are found only in thromboembolic stroke patients and not in those with lacunar strokes.<sup>40</sup> In addition, one study showed that higher levels of VCAM-1 at admission were associated with the risk of recurrence or death within 1 year after the ischemic event.<sup>45</sup> Similarly, an association was found between higher VCAM-1 levels 2–3 weeks after the event and a worse 3-month outcome in ischemic stroke patients.<sup>44</sup> The interaction between VLA-4 and VCAM-1 is crucial for the transmigration of leukocytes to the site of injury; for that reason, the inhibition of this interaction by specific antibodies seems to be a promising strategy to combat stroke.<sup>48</sup> In fact, several experimental studies have analyzed the effect of VCAM-1/VLA-4 axis blockade in stroke animal models. Although not all studies obtained the same results, the vast majority have reported beneficial effects of this blockade. In 2001, two independent studies reported for the first time that VLA-4 blockade by antibodies reduced the infarct size and improved the neurological function after a transient occlusion in rats.<sup>51,52</sup> However, the results obtained in mouse models are not as consistent. VLA-4 blockade produced a reduced infarct size and improved neurological function only when the antibody was administered after 30–45 min of occlusion in a tMCAO mouse model,<sup>46,48,49</sup> and not after 60 min of tMCAO<sup>46</sup> or after pMCAO.<sup>48</sup> The discrepancies obtained between the studies are probably due to important differences in the design and methodology. To find more robust results, a group of researchers from six different centers performed a multicenter and randomized preclinical trial to explore the efficacy of VLA-4 blockade in two distinct mouse models of stroke. The

results of this study revealed that the treatment with anti-VLA-4 significantly reduced infarct volume after distal pMCAO, which produced small cortical infarctions. However, after 60 min of tMCAO, the procedure that produces larger lesions, the blockade of VLA-4 did not reduce infarct size, suggesting that the efficacy of this treatment depends on infarct severity and localization.<sup>47</sup> Even with this information in mind, the efficacy of VLA-4 blockade has also been studied in stroke patients. Natalizumab is a recombinant immunoglobulin G4 monoclonal antibody that binds to the alpha subunit of the VLA-4 integrin to block the leukocyte transmigration. Last year, the ACTION trial, a double-blind phase II study exploring the safety and efficacy of natalizumab in reducing the infarct volume in acute ischemic stroke was concluded. A total of 161 stroke patients were randomly assigned to 300 mg intravenous natalizumab ( $n = 79$ ) or placebo ( $n = 82$ ). It revealed that a single dose of natalizumab administered within the first 9 h after the onset of symptoms was not able to reduce infarct volume. However, functional outcome at 30 and 90 days was improved in patients receiving natalizumab.<sup>50</sup> An ongoing ACTION 2 trial [ClinicalTrials.gov identifier: NCT02730455] with 270 participants is currently testing the efficacy of this treatment using a high (600 mg) or a low dose (300 mg), given that the ACTION trial revealed that patients with higher serum natalizumab concentrations showed better clinical outcomes. The results from the ACTION 2 trial are still not available, although a press release on February 2018 from the company behind the study points to the failure of the trial since the primary and secondary efficacy endpoints were not met.

P-selectin is an adhesion molecule expressed on the surface of activated endothelial cells and platelets that mediates the rolling of leukocytes.<sup>138</sup> In reference to changes in the circulating levels of this protein after ischemic stroke, various studies have reported that ischemic stroke patients have higher levels of P-selectin than controls.<sup>23,24,138-140</sup> Therapies targeting P-selectin have also been shown as effective in various experimental studies. Connolly and colleagues demonstrated for the first time that the blockade of P-selectin after tMCAO in mice reduced the infarct volume and improved the functional outcome.<sup>141</sup> Similarly, a reduction of the infarct volume was also reported after anti-P-selectin administration following pMCAO in rats.<sup>143,144</sup> However, Goussev and

colleagues found that the administration of the anti-P-selectin antibody upon reperfusion after tMCAO in rat did not reduce the infarct size.<sup>142</sup> Thus, further studies need to be performed in translational experimental models in order to confirm the potential role of this protein as a therapeutic target, but the results obtained for the moment do seem promising.

#### *Damage-associated molecular patterns*

DAMPs are endogenous molecules released from injured cells that can initiate or maintain the inflammatory response. A well-known DAMP implicated in ischemic brain injury is a protein named high-mobility group box 1 (HMGB1), a nuclear protein that functions as a nucleosome stabilizer and transcription factor. In addition to this, HMGB1 is able to activate endothelial cells, increasing VCAM-1, ICAM-1 and E-selectin expression, and consequently, allowing leukocyte extravasation. Moreover, HMGB1 can act itself as a pro-inflammatory cytokine because it is secreted by activated immune cells and can mediate the systemic inflammatory response.<sup>15</sup> In reference to the circulating levels of this protein in ischemic stroke patients, distinct studies have reported an elevation of HMGB1 after stroke in comparison with controls.<sup>86,145-147</sup> Moreover, increased plasma levels of HMGB1 have been described as associated with a poor functional outcome within 1 month or 1 year after stroke.<sup>86,148</sup> However, Schulze and colleagues reported no association between HMGB1 levels and outcome or infarct size.<sup>147</sup> Experimental studies on a tMCAO rat model have reported that the intracerebroventricular injection of HMGB1 increased the severity of infarction, whereas the administration of anti-HMGB1 reduced the infarct size, improved the neurological function<sup>149</sup> and decreased the edematous area.<sup>170</sup> Although the results obtained seem promising, more experimental studies using different animal models are still needed to confirm the findings reported.

#### *Matrix metalloproteinases*

Matrix metalloproteinases (MMPs) are zinc-binding proteolytic enzymes that remodel the extracellular matrix. In addition to this, MMPs are also involved in different biological processes such as the cleavage of cell receptors, the release of death signals, the activation of inflammatory mediators, cell proliferation and apoptosis.<sup>12</sup>

MMPs are secreted by neurons, glial and endothelial cells, as well as by infiltrating leukocytes as inactive enzymes; thus, they must be cleaved by other proteases to become active.<sup>171</sup> The most studied MMPs in the field of ischemic stroke are MMP-9 and MMP-2, which are gelatinases that specifically degrade type IV collagen, laminin, and fibronectin, the major components of the basal lamina around cerebral blood vessels.<sup>172</sup> In fact, a huge amount of studies have reported increased circulating levels of MMP-9 in ischemic stroke patients within 24 h after the event in comparison with controls<sup>86–93</sup> and even stroke mimics.<sup>94–96</sup> However, these differences in MMP-9 plasma levels were not observed in one study comparing controls with stroke patients (including both the ischemic and hemorrhagic stroke subtypes).<sup>97</sup> In reference to MMP-2, two independent studies have reported decreased levels of this protein in ischemic stroke patients when compared with controls.<sup>93,102</sup> However, the lack of differences between patients and controls has also been reported.<sup>92</sup> In addition to this, higher MMP-9 levels have also been associated with larger infarct volumes and worse 3-month outcomes, including mortality.<sup>21,68,86,89,93,98,99,102–106</sup> Furthermore, distinct studies have reported that increased levels of MMP-9 at admission (before rt-PA administration) are associated with hemorrhagic transformation following the thrombolytic treatment.<sup>92,107–110</sup> For that reason, MMP-9 levels at admission could work as a good biomarker to predict this secondary complication. In reference to experimental models of ischemic stroke targeting MMPs, a highly selective inhibitor that targets both MMP-2 and MMP-9 [(4-phenoxyphenylsulfonyl) methylthiirane (SB-3CT)] has been tested. Gu and colleagues explored whether the administration of SB-3CT after transient ischemia in mice could be neuroprotective. They found that the blockade of MMP-2 and MMP-9 produces a decrease in the infarct volume in comparison with vehicle-treated controls and ameliorates the functional outcome.<sup>111</sup> Moreover, the same results were obtained when administering SB-3CT after pMCAO in mice.<sup>112</sup> Thus, all experimental studies performed so far seem promising, although further studies need to be undertaken before any conclusions can be drawn. Moreover, no one has deeply studied the effect of specifically blocking MMP-9 or MMP-2, which may be interesting in terms of revealing the specific role of each protein in stroke pathophysiology.

### Annexins

Annexins are a family of proteins involved in vesicle transport, membrane scaffolding, apoptosis, proliferation, differentiation and inflammation. Annexin A1 (ANXA1), previously known as lipocortin 1, is the only annexin that has been studied in the context of stroke as a possible biomarker and therapeutic target. There is an increasing evidence of the role of ANXA1 in different anti-inflammatory processes, such as the regulation of macrophage phagocytosis and neutrophil migration through the inhibition of neutrophil adhesion to the endothelium.<sup>173</sup> One study exploring a small cohort of patients has reported no differences in the ANXA1 plasma levels between ischemic stroke patients, controls and patients with stroke-mimicking conditions.<sup>150</sup> In addition, studies in animal models of cerebral ischemia found that the intracerebral administration of ANXA1 reduced the infarct size after pMCAO in rats, whereas the inhibition of ANXA1 by antibodies produced larger lesions in the same model.<sup>151</sup> Hence, only one study has explored the effect of modulating this protein in experimental models; thus, it is necessary to perform more studies in order to confirm these promising results. Moreover, it would be interesting to explore the potential role of this protein as a stroke biomarker in large cohorts of patients.

### Limitations and future steps

As described earlier, the understanding and modulation of the postischemic inflammatory response is gaining interest among scientists in the field of stroke. In recent years, several studies have explored the potential of inflammatory molecules to become stroke biomarkers or therapeutic targets. Although the majority of the results obtained seem promising, there also exist contradictory results and lack of robustness in certain cases. However, it is important to bear in mind that ischemic stroke is a complex disease in which several biological processes are altered, and different molecules may interact. Moreover, the inflammatory response triggered after the ischemic event shows dynamic behavior; thus, the implicated molecules may have different roles depending on the moment they act. For that reason, studies exploring the role of some inflammatory proteins as stroke biomarkers may not be as robust as expected, mainly due to the differences in the time of measurement of the proteins among exploratory studies, and the limited sample size of several

studies. In addition to this, almost all studies assaying the potential usage of inflammatory proteins as biomarkers for stroke diagnosis are comparing circulating levels of these proteins between ischemic stroke patients and healthy controls; this experimental design is far from reality and does not ensure the efficacy of these biomarkers in stroke diagnosis. Studies exploring acutely the differences in circulating concentrations of these proteins between strokes and stroke-mimicking conditions such as seizures, headaches or brain tumors, seem thus necessary, as the inflammatory response is also altered in some of these mimics, who are the real confounders in stroke diagnosis.

In addition, the pharmacological modulation of the inflammatory response seems to be a promising strategy for stroke treatment as reported in several experimental studies. However, the existence of different ischemic stroke models and differences in experimental designs, including different administration times and protocols, may generate opposite results among studies that are also influenced by the dynamic nature of the inflammatory processes following stroke. To make feasible the translation of these treatments to the clinical setting, it seems necessary to adjust the experimental designs to a situation closer to the pathophysiology and timing occurring in humans. Moreover, the application of the current guidelines for preclinical studies in stroke would add great value to the search for new therapies.<sup>174,175</sup>

The results summarized in this review underlined a plausible use of inflammation-related proteins as blood biomarkers, therapeutic targets and even surrogate biomarkers of the efficacy of therapeutic strategies directed against the same target molecule. This will definitely provide a more personalized management of stroke patients. For that reason, a better characterization and understanding of the stroke pathophysiological processes such as inflammation is necessary to overcome the current limitations, as well as a deeper understanding of the importance of precise times in the diagnosis and treatment response in stroke.

The current context of stroke, in which reperfusion therapies are the only approved treatments, seems ideal for the exploration of new biomarkers and therapeutic targets, such as those described here. In an ideal scenario, some of these inflammatory-related molecules might be measured by future point-of-care devices in order to rapidly diagnose ischemic stroke. This might help increasing the

number of patients who can benefit from the actual reperfusion therapies. Moreover, since many stroke patients do not respond properly to these therapies, the pharmacological modulation of inflammatory-related molecules as a complementary therapeutic tool could be an interesting approach to improve stroke patients' management and consequently, their outcome.

### Conclusion

Despite improvements in ischemic stroke management, appropriate diagnostic tools and therapies beyond vascular reperfusion are still missing. There is an urgent need for pharmacological agents to reduce the ischemic damage. Inflammation is a key process involved in the pathophysiology of stroke and its modulation seems a promising strategy for neuroprotection. Various studies have demonstrated that inflammatory molecules could work as biomarkers for stroke diagnosis or prognosis. Moreover, numerous animal model studies have shown that pharmacological modulation of these inflammatory molecules can reduce infarct size and improve functional outcome. However, the translation from bench to bedside has not been yet accomplished. To that end, a better understanding of the postischemic inflammatory response would increase the chances to find new diagnostic and therapeutic strategies.

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### Conflict of interest statement

The authors declare that there is no conflict of interest.

### ORCID iD

Laura Ramiro  <https://orcid.org/0000-0002-4121-4609>



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